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# Role of Fungi in the Heating of Moist Wheat[1]

By Edward P. Carter, formerly *assistant physiologist*, and George Y. Young, formerly *assistant pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*[2]

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## INTRODUCTION

The average annual loss due to heating of wheat in commercial storage in the United States has been estimated at about 2 million dollars (*13*),[3] the amount varying greatly from year to year and depending principally on the moisture content of the grain when it is harvested or put into storage. The loss in farm bins and country elevators has not been determined, but doubtless it greatly exceeds that in commercial storage.

It has long been known that heating may develop in sound wheat stored with a moisture content in excess of 14 to 14.5 percent, or even less. Furthermore, it has often been observed that, in the absence of insects, heating in damp grain occurs at about the same moisture content at which certain common molds invariably present on threshed wheat develop and that mold growth often accompanies heating.

[1] The studies here reported were a part of the investigations on grain storage on the farm, supported by funds under an allotment from the Special Research Fund of the Bankhead-Jones Act of June 29, 1935.

[2] The writers wish to acknowledge the aid rendered by A. G. Johnson, S. C. Salmon, J. H. Martin, and Charles Thom through their continued interest and advice during the course of these investigations.

[3] Italic numbers in parentheses refer to Literature Cited, p. 24.

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Accordingly, it has been suggested by a number of investigators that the growth of fungi is a principal cause of heating. Considerable experimental data supporting that view have been accumulated. On the other hand, there is some evidence to the contrary. In connection with the extensive grain-storage experiments conducted by the United States Department of Agriculture and cooperating State agricultural experiment stations, the results of which are reported elsewhere (*21*), it seemed desirable that the role of fungi be further investigated. The experiments reported in this circular were designed for that purpose.

## REVIEW OF LITERATURE

A comprehensive review of the literature on heat production, especially spontaneous combustion, in stored organic materials was published by Browne (*6*) in 1929. More recently Ramstad and Geddes (*34*) reviewed the literature on respiration relating to the heating of grain in storage. Hence, it seems desirable to mention only those contributions that are especially pertinent to the present study.

Cohn (*12*) appears to have been the first to distinguish between the heat of respiring grain and that produced by the action of associated micro-organisms. He found that well-moistened barley placed in an apparatus designed to prevent the loss of heat and moisture and permit the exchange of carbon dioxide and oxygen heated to about 65° C. There was a slight pause at about 40°, after which heating continued. Since the young plants were killed at about 40°, Cohn believed that the heating at first was caused by the respiration of the germinating grain and the later heating by the activity of micro-organisms, principally *Aspergillus fumigatus*.

A number of investigators (*3*, *7*, *14*, *16*, *17*, *19*, *20*, *24*, *27*, *28*, *30*, *33*, *34*, *38*, *39*, *43*) have supported Cohn's theory wholly or in part. Others (*1*, *2*, *13*) have attributed heating mostly or entirely to the respiration of the grain itself.

Many investigators (*4*, *9*, *10*, *11*, *12*, *15*, *23*, *25*, *31*, *35*, *36*) have reported the presence of micro-organisms on seeds of various kinds.

That the particular organism found may depend on the moisture content was shown by Thom and LeFevre (*41*) and Koehler (*22*). Thom and LeFevre (*41*) found that *Aspergillus repens*, a member of the *glaucus* group, would grow on corn meal at moisture contents between 13 and 15 percent. Members of the *A. flavus* group appeared at 16 percent, and numerous fungi including *A. niger*, *A. candidus*, and species of *Penicillium* and *Actinomyces* appeared at 18 to 20 percent.

Koehler (*22*) inoculated surface-sterilized corn containing various quantities of moisture with pure cultures of a number of fungi. Each species was found to have a critical minimum-moisture requirement. *Aspergillus glaucus* developed satisfactorily at 14 percent moisture; but *A. flavus* would not grow until a moisture content of 18 percent was reached, and *A. niger* required a moisture content of 19.1 percent.

Other conditions of the environment are undoubtedly important. Bekker (*5*), for example, found that species of *Aspergillus* and *Penicillium* predominated under aerated storage conditions, whereas *Oospora variabilis* and *Micrococcus pyogenes* var. *albus* were most abundant in closed containers. Morgenthaler (*31*) reported bacteria, chiefly *Bacterium herbicola*, but no fungi on healthy grain. Musty grain, on the other hand, yielded few bacteria but many fungi, prin-

cipally species of *Penicillium*. An unsuccessful attempt was made to determine which of these fungi was responsible for the musty odor.

## MATERIAL AND METHODS

A serious difficulty in determining the role of fungi in the heating of wheat is the apparent impossibility of sterilizing wheat without killing it or seriously altering its metabolism. If such sterilization were possible, the heat due to respiration of fungi and that due to the respiration of the wheat itself could be measured separately. So far as known to the writers, unharmful sterilization has not been reported, but by the use of sulfa drugs Milner (*29*) has made noteworthy progress in distinguishing respiration of fungi and of the infested wheat kernels.[4] Despite several attempts to make such a separation by the use of various methods, the writers were not successful. They, therefore, attempted an indirect approach to the problem.

The survey to determine the prevalence of fungi on wheat consisted of the identification of those on 355 samples of soft red winter, hard red winter, and hard red spring wheats (*Triticum aestivum* L. (*T. vulgare* Vill.)) obtained from the several locations in which the previously mentioned storage experiments (*21*) were being conducted.

Isolations for the identification of fungi were made on potato-dextrose agar plates, three types of plating being made from each sample as follows: (1) Plating unsterilized kernels directly onto the agar; (2) plating directly onto the agar kernels that had been surface-sterilized by washing in 70-percent alcohol for 1 minute and then in sodium hypochlorite solution containing 4 percent of available chlorine for 10 minutes; and (3) washing a known number of kernels in sterile water and applying to the plates suitable spore dilutions varying from 1 : 1 to 1 : 10,000. All plates were incubated at room temperatures (25° to 33° C.) for 5 days, colony counts were then made, and streak transfers from individual colonies were made to fresh sterile potato-dextrose agar slants in test tubes. These were incubated at room temperature for 3 days and afterward kept at 5° for future use.

Spore-load determinations were made on samples representing soft red winter, hard red winter, and hard red spring wheats by taking small samples, counting the number of kernels, washing the kernels in 15 cc. of distilled water for 1 minute, and decanting and saving the supernatant liquid. The suspensions from 10 to 15 washings were

[4] After the manuscript for this circular was almost completed, Milner (*29*), by the use of sulfa drugs, succeeded in distinguishing the respiration of moist wheat kernels themselves from that of the fungi active on them. He used Regent wheat (94 percent germination, with 20 percent moisture) and incubated it in respirometers at 30° C. Of the 11 sulfa compounds used, finely ground sulfanilamide, dusted on the moist seed at the rate of 1 : 250 by weight, gave the best results. The daily respiratory rate of the dusted and undusted (control) samples was determined for 11 days. On the first day the respiratory rate was the same for the treated and untreated samples. Thereafter the respiratory rate for the treated sample remained essentially constant, but that for the untreated sample increased conspicuously after the molds had started growing, until on the eleventh day it was 428 mg. of carbon dioxide per 100 gm. of dry matter for 24 hours, as compared with only 43 mg. for the treated sample. At the end of the eleventh day the treated sample germinated 84 percent whereas the untreated sample germinated only 10 percent. Milner's results thus furnish additional evidence that the great increase in the respiratory rate of moist grain, and therefore the heating, is caused by the activity of fungi.

pooled, centrifuged, and diluted to some known volume consistent with countability, depending on the relative spore concentration as determined visually. The spore suspension was then shaken vigorously, a small quantity was pipetted into a Levy-Hussar counting chamber, counts were made in duplicate and averaged, and the number of spores per kernel was calculated.

An estimate of the random errors was made on 100 aliquots of a sample of wheat containing approximately 8,000,000 spores per kernel and also on 40 aliquots from a commercial lot of No. 1 wheat having a spore load of about 6,000 spores per kernel. The standard deviations and coefficients of variability were found to be about 1,250.000 and 17 percent for the high-spore-load samples and 6,850 and 114 percent for the low-spore-load samples.

Experiments were made to determine the role of fungi in heating. Naturally infested, viable wheat and dead wheat were stored at various moisture levels in cotton-stoppered and in sealed quart-capacity glass vacuum flasks, with and without treatment by various chemicals. The flasks were fitted with thermocouples and were kept in the laboratory at room temperatures until heating appeared to have reached a maximum. Before the wheat was placed in the flasks, all samples were brought to the desired moisture content, thoroughly mixed, and allowed to stand at room temperature for 36 hours. During the experimental period the temperatures were recorded daily. The moisture content of the wheat, the germination, and the spore load of each sample were determined at the close of each experiment. In some experiments spore loads were determined at intervals by taking samples from the flasks.

Wheat was killed in some cases by soaking it in water for 1 hour and then placing it in a hot-air oven for 6 hours at 120° C. and in other cases by exposure in sealed containers to carbon disulfide ($CS_2$) or chloropicrin ($CCl_3NO_3$; trichloronitromethane) for 24 hours.

As recently pointed out by Carlyle and Norman (*7*), data obtained in this way are subject to certain limitations, because at the higher temperatures the flasks lose heat at a faster rate than it is evolved. In the present study this difficulty was avoided in part by including untreated controls in each experiment.

Another difficulty is the variation in the rate of heat loss in different flasks, which in some cases was very material. In order to prevent serious errors, the rate of cooling of each flask was determined and only those with rates substantially alike were used in any experiment.

Oxygen respiration was determined in Warburg respirometers fitted with flasks designed for small grain. These determinations were limited to wheat that was first sterilized and killed and then inoculated with certain organisms which the preceding studies indicated were associated with heating. An uninoculated sample was run as a control. Control and inoculated samples were replicated four times in each experiment. For comparison, samples inoculated with the same organisms were placed in the glass vacuum flasks previously described and kept at room temperature for observations as to heating. These samples were replicated four times in each experiment.

The wheat for these studies was prepared by bringing it to a moisture content of about 20 percent by the addition of water. It was then allowed to stand at room temperature for 3 days, during which it was

frequently shaken and passed through a Boerner sampler to insure an even moisture distribution. Quart samples were removed, placed in cotton-stoppered 2-liter Erlenmeyer flasks, which were then autoclaved for 30 minutes at 20 pounds' pressure. The resulting sterile dead wheat was again allowed to stand at room temperature for 3 days, with frequent shaking to insure an even distribution of moisture. At the end of this period the flasks were divided into five groups, four of which were inoculated with spores of a pure culture of the following: *Aspergillus candidus* Lk. ex Fr., *A. niger* v. Tiegh., *A. flavus* Lk. ex Fr., and *A. glaucus* Lk. ex Fr. The fifth group was used as a sterile control. The inoculated wheat was shaken thoroughly to insure an even distribution of spores and was then transferred aseptically to previously numbered and sterilized, cotton-stoppered quart-capacity vacuum flasks. In a like manner uninoculated samples were transferred to vacuum flasks to serve as controls.

Small portions, each of about 10 gm., were withdrawn from two flasks of each group and placed in sterile Warburg flasks fitted with a cup in which was placed 2 cc. of a 15-percent solution of potassium hydroxide for the absorption of evolved carbon dioxide. The flasks were then attached to manometers and mounted in a water bath at 28° C.

Respiration readings were taken daily at several intervals for 17 days in the first experiment and 18 days in the second. The results were expressed as cubic millimeters ($mm.^3$) of oxygen, at standard temperature and pressure, consumed per hour per gram of wheat (dry weight) and were reported as the daily averages of the individual readings.

A record of the temperature in each vacuum flask was made by recording the temperature daily by a thermocouple and expressing the result as temperature increment in degrees centigrade, using the temperature of an empty flask as a base for the increment calculation. This record was reported as the daily temperature increment.

At the end of the experimental period the flasks were removed from the manometers, the contents photographed, and spore loads determined. Spore-load and fat-acidity determinations were also made on the samples in the vacuum flasks at the end of the experiment. Fat acidity was determined by the method described by Zeleny (*45*), and the results were reported as the number of milligrams of potassium hydroxide necessary to neutralize the free fatty acids from 100 gm. of grain (dry basis).

## EXPERIMENTAL RESULTS

### Fungi Commonly Present on Samples of Commercial Wheat

Fungi were isolated and in part identified for 355 samples of soft red winter, hard red winter, and hard red spring wheats. In order of their most frequent occurrence on unsterilized kernels they were *Aspergillus glaucus*, *A. flavus*, *A. candidus*, *Penicillium expansum* Thom, other species of *Penicillium*, *Rhizopus* spp., *Mucor* spp., *A. niger*, *Alternaria* spp., *Aspergillus repens* (Cda.) DBy., *A. tamarii* Kita, *Chaetomium* spp., *A. fumigatus* Fres., *A. clavatus* Desm., *Fusarium* spp., *Cladosporium* spp., *Helminthosporium* spp., and one sterile fungus. Examination revealed the presence of *Actinomyces*, but individual species were not isolated.

Surface-sterilized kernels from the same samples yielded the following, listed in the order of their most common occurrence: *Alternaria* spp., *Aspergillus glaucus*, *Chaetomium* spp., *A. niger*, *A. flavus*, *A. terreus* Thom, and *Penicillium* spp.

Most of the above-named fungi could be isolated from every sample, and no clear-cut data that would indicate a difference in the microfloras of the hard and soft wheats were obtained. Likewise, it was impossible to establish any relation between the prevalence of any particular fungus or group of fungi and the geographic origin of the samples.

## Spore Loads of Stored Wheat

Samples of soft red winter wheat from 10 commercial sources, 18 experimental bins in Maryland, and 1 bin of hard red spring wheat in North Dakota were taken in July and August 1935 and examined for spore loads. The experimental bins were sampled at irregular intervals afterward, and these samples also were examined. None of the commercial lots contained more than 12 percent of moisture, but most of the experimental wheat contained too much moisture to store safely. The spore loads of these various samples, together with the official grade, the grading factor, the moisture content, and the percentage of germination, are given in table 1. Fat-acidity determinations were made for some of the samples.

The spore loads of the commercial samples ranged from 3,320 to 56,665 spores per kernel, all but 3 having less than 20,000. The spore loads of the wheat from the experimental bins were somewhat higher, ranging from 19,166 to 61,665 per kernel after 1 or 2 days in storage. All the experimental bins except B–9 and B–18 were filled with high-moisture wheat. In 15 of the 18 bins there was a marked increase in spore load, the number in bin B–8 at the last sampling date being 910,900 spores per kernel. The moisture content of the wheat in bins B–10 and B–11, in which there was no significant increase in spore loads, was only slightly higher than is considered safe for storage. Wheat in all these bins deteriorated during storage, as shown by market grade, decreased viability, or increased fat acidity. Fat acidity (not shown in the table) was determined for the first and last samples from bins B–1, B–2, B–10, B–17, and F–10. There was a marked increase (42 units) for bin B–1, a moderate increase (5 to 8 units) for bins B–2, B–10, and B–17, and no increase for F–10.

The wheat in bin B–9 contained only 11.4 percent moisture after 1 day in storage. It did not deteriorate, but the spore load increased slightly. The spore load of the wheat in bin B–18, which contained only 11.3 percent moisture after 1 day in storage, increased during the first 112 days in storage and then decreased.

Altogether, the limited data are in accord with the general belief that wheat normally carries a sufficient spore load to initiate fungus development whenever conditions are favorable and that in high-moisture wheat the spore loads tend definitely to increase with storage during the summer months.

## Relation of Fungi to Heating of Moist Wheat

Before any studies of heating were undertaken, the quart glass vacuum flasks to be used were filled with dry wheat heated to about 60° C. and allowed to cool, the temperature in each being recorded

TABLE 1.—*Relation between market grade and condition and germination and spore load of wheat in commercial and experimental storage bins*

| Sample No. [1] | Time in storage | Official grade of sample | Grading factor | Germination | Moisture | Spore load (spores per kernel) |
|---|---|---|---|---|---|---|
| | *Days* | | | *Percent* | *Percent* | *Number* |
| 1 | 0 | No. 1 | | 99 | 10.8 | 56,665 |
| 2 | 0 | do | | 98 | 11.5 | 39,165 |
| 3 | 0 | do | | 98 | 12.0 | 5,550 |
| 4 | 0 | do | | 98 | 11.8 | 5,700 |
| 5 | 0 | do | | 96 | 11.4 | 8,500 |
| 6 | 0 | do | | 93 | 10.8 | 3,320 |
| 7 | 0 | do | | 92 | 11.3 | 6,100 |
| 8 | 0 | do | | 96 | 11.5 | 4,875 |
| 9 | 0 | do | | 96 | 11.1 | 4,800 |
| 10 | 0 | do | | 98 | 12.0 | 20,000 |
| B–1 | 2 | Sample | Moisture | 87 | 16.4 | 30,000 |
| | 27 | do | Odor (sour); moisture | 12 | 16.2 | 219,500 |
| | 27 | do | do | 6 | 16.0 | 387,500 |
| | 36 | do | do | 6 | 16.0 | 375,000 |
| | 36 | do | Odor (sour) | 4 | | 709,165 |
| | 45 | do | Moisture | 1 | 15.9 | 345,962 |
| | 45 | do | Odor (sour) | 1 | | 365,000 |
| | 69 | do | do | 11 | 14.8 | 423,000 |
| B–2 | 2 | Sample | Moisture | 87 | 16.4 | 30,000 |
| | 26 | do | Odor (sour); moisture | 14 | 16.2 | 400,000 |
| | 26 | do | do | 25 | 16.2 | 315,000 |
| | 35 | do | Damage; moisture | 2 | 16.3 | 345,000 |
| | 35 | do | Odor (sour); moisture | 8 | 16.3 | 390,832 |
| | 46 | do | do | 0 | 15.6 | 177,500 |
| B–3 | 1 | Sample | Moisture | 90 | 16.5 | 26,665 |
| | 32 | do | Odor (sour); moisture | 20 | 16.1 | 294,160 |
| | 32 | do | do | 27 | 16.1 | 290,000 |
| | 40 | do | do | 18 | 16.2 | 680,000 |
| | 40 | do | do | 16 | 16.2 | 300,000 |
| | 46 | do | do | 11 | 15.9 | 320,000 |
| B–4 | 1 | Sample | Moisture | 90 | 16.5 | 26,665 |
| | 31 | do | Odor (sour); moisture | 23 | 16.2 | 240,000 |
| | 31 | do | Odor (sour) | 12 | | 277,500 |
| | 39 | do | Odor (sour); moisture | 20 | 16.1 | 868,325 |
| | 39 | do | Odor (sour) | 15 | | 215,000 |
| | 46 | do | Odor (sour); moisture | 2 | 16.1 | 200,000 |
| B–5 | 2 | Sample | Moisture | 94 | 16.5 | 23,000 |
| | 29 | do | Odor (sour); moisture | 24 | 16.1 | 442,500 |
| | 29 | do | do | 18 | 16.1 | 286,665 |
| | 38 | do.[2] | do | 22 | 16.1 | 183,332 |
| | 38 | do.[2] | do | 9 | 16.1 | 249,165 |
| | 46 | do.[2] | do | 5 | 15.9 | 174,165 |
| B–6 | 2 | Sample | Moisture | 94 | 16.5 | 23,250 |
| | 69 | do | Odor (sour) | 0 | 15.3 | 467,500 |

See footnotes at end of table.

TABLE 1.—*Relation between market grade and condition and germination and spore load of wheat in commercial and experimental storage bins*—Continued

| Sample No. [1] | Time in storage | Official grade of sample | Grading factor | Germination | Moisture | Spore load (spores per kernel) |
|---|---|---|---|---|---|---|
| | *Days* | | | *Percent* | *Percent* | *Number* |
| B–7 | 2 | Sample | Moisture | 83 | 16.4 | 29,165 |
| | 34 | do | Odor (sour); moisture | 48 | 16.4 | 571,665 |
| | 34 | do | do | 48 | 16.4 | 256,665 |
| | 42 | do | do | 9 | 16.4 | 305,000 |
| | 42 | do | do | 11 | 16.0 | 303,333 |
| | 69 | do [3] | Odor (sour) | 1 | 15.0 | 485,000 |
| B–8 | 2 | Sample | Moisture | 83 | 16.4 | 29,165 |
| | 69 | do | Odor (sour) | 41 | 15.2 | 910,900 |
| B–9 | 1 | No. 2 | Test weight | 88 | 11.4 | 26,665 |
| | 112 | do | do | 83 | 12.5 | 33,333 |
| | 176 | do | do | 91 | 12.8 | 38,332 |
| B–10 | 1 | No. 4 [3] | Test weight; moisture | 92 | 15.0 | 27,500 |
| | 112 | do [3] | do | 54 | 14.3 | 28,250 |
| | 176 | do [3] | Damage; moisture | 56 | 14.5 | 25,832 |
| B–11 | 1 | Sample | Moisture | 95 | 15.7 | 24,165 |
| | 108 | No. 3 [3] | Test weight; moisture | 13 | 15.4 | 29,165 |
| | 172 | Sample | Moisture | 61 | 15.6 | 28,332 |
| B–12 | 1 | Sample [4] | Moisture | 92 | 18.0 | 61,665 |
| | 89 | do [3 4] | Damage; moisture | 15 | 14.4 | 54,250 |
| B–13 | 2 | Sample | Moisture | 91 | 16.4 | 42,500 |
| | 118 | No. 3 [3] | Test weight; moisture | | 15.3 | 157,500 |
| | 182 | Sample | Moisture | 68 | 15.7 | 51,665 |
| B–14 | 3 | Sample | Moisture | 88 | 16.4 | 36,665 |
| | 118 | No. 3 [3] | Test weight; moisture | | 15.5 | 556,650 |
| | 182 | Sample | Moisture | 44 | 15.8 | 141,665 |
| B–15 | 1 | Sample [5] | Moisture | 98 | 17.9 | 32,500 |
| | 9 | do [5] | Damage | 33 | 14.2 | 451,650 |
| B–16 | 1 | Sample | Moisture | 93 | 15.7 | 25,822 |
| | 108 | No. 3 [3] | Test weight; moisture | | 15.0 | 78,332 |
| | 172 | do [3] | do | 61 | 15.3 | 58,500 |
| B–17 | 1 | No. 4 [3] | Test weight; moisture | 94 | 15.0 | 19,166 |
| | 112 | do [3] | Damage; moisture | 84 | 14.2 | 33,333 |
| | 176 | do [3] | do | 87 | 14.6 | 64,165 |
| B–18 | 1 | No. 2 | Test weight | 82 | 11.3 | 19,166 |
| | 112 | | | 79 | | 79,165 |
| | 176 | No. 2 | Test weight | 86 | 12.7 | 29,165 |
| F–10 | 0 | No. 4 [3] | Test weight; moisture | 68 | 15.1 | 55,000 |
| | 57 | Sample [3] | Odor (sour); moisture | 59 | 14.8 | 70,000 |

[1] Letters indicate location of the bins; B, for College Park, Md.; F, for Fargo, N. Dak.
[2] Wheat treated with lime.
[3] Also tough.
[4] Air-dry peat moss mixed with wheat at rate of 3.2 pounds per bushel.
[5] Air-dry peat moss mixed with wheat at rate of 2.1 pounds per bushel.

by a thermocouple as cooling progressed. Data for 45 typical flasks are presented in figure 1. There was a marked variation in the rate of loss of heat in the various flasks. About 10 percent of them, most but not all of which had been used in earlier experiments, were found to be inadequately insulated, as illustrated by the temperature curves for the two defective flasks in figure 1. These flasks were discarded. Considerable variation was noted, even in those that were considered acceptable.

Experiments designed to measure and correlate the rate of heat production in moist wheat in vacuum flasks stored at room tempera-

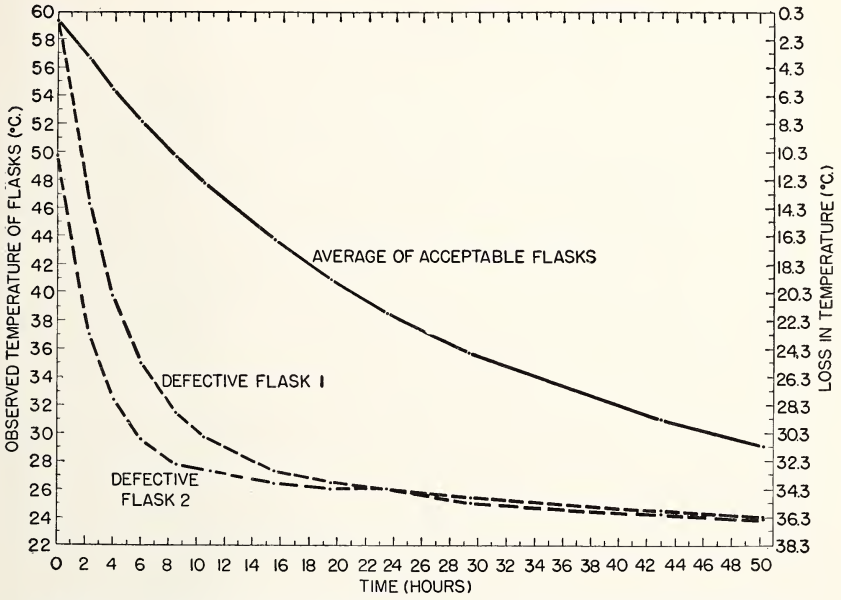


FIGURE 1.—Rates of cooling dry, hot wheat in quart glass vacuum flasks.

tures with the growth of certain fungi, as indicated by spore loads, have provided strong circumstantial evidence that micro-organisms are responsible for most of the heating observed in stored grain.

The results of these tests with treated and untreated, viable and dead wheat stored in cotton-stoppered flasks are presented in tables 2 and 3. The temperature and spore-load changes observed during the course of the experiment are presented in detail for viable wheat untreated and treated with six drops of chloropicrin per quart and for dead wheat killed by moist heat and by saturation with carbon disulfide (figs. 2 and 3). The results obtained with moist wheat held in sealed vacuum flasks are not given in tables 2 and 3, because heating was not observed in any treatment or in the untreated control samples. Wheat in the sealed flasks was found to deteriorate rapidly at the higher moisture levels, and a special study, reported elsewhere (*8*), was made of the effect of storing wheat in sealed containers. Comparison between untreated wheat stored in cotton-stoppered vacuum flasks and in sealed flasks is shown in figures 2 and 3 (lines 2 and 5).

Under the conditions of the experiments herein reported, the rate of heat production in untreated samples of moist wheat was greatest

for the first 20 days, thereafter proceeding more slowly until the end of the experimental period (fig. 2, line 2).

In all cases when heat production was moderate or severe, the principal fungus isolated was *Aspergillus candidus*. Spore production on wheat heavily infested with this fungus was very rapid during the first 15 days, and thereafter it fell gradually until the experiment was discontinued (fig. 3, line 2).

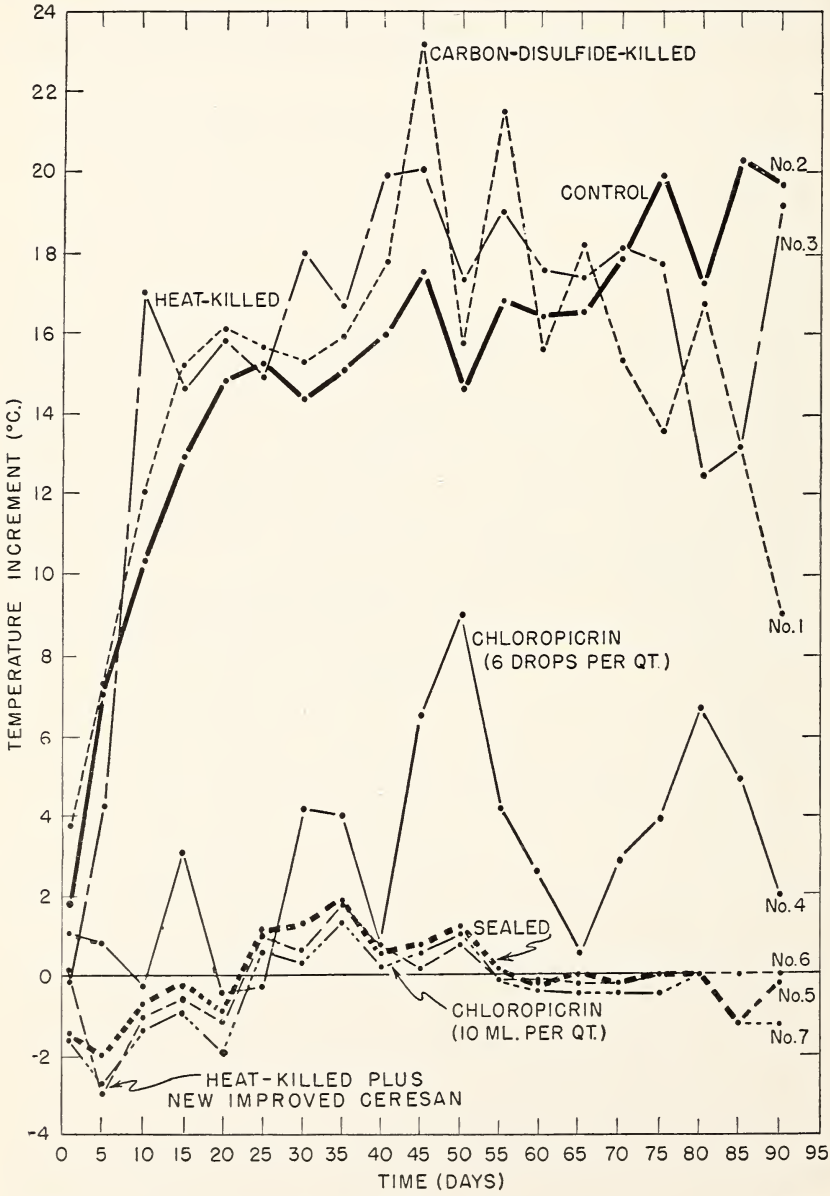


FIGURE 2.—Temperature changes observed in moist wheat stored in quart glass vacuum flasks after various treatments.

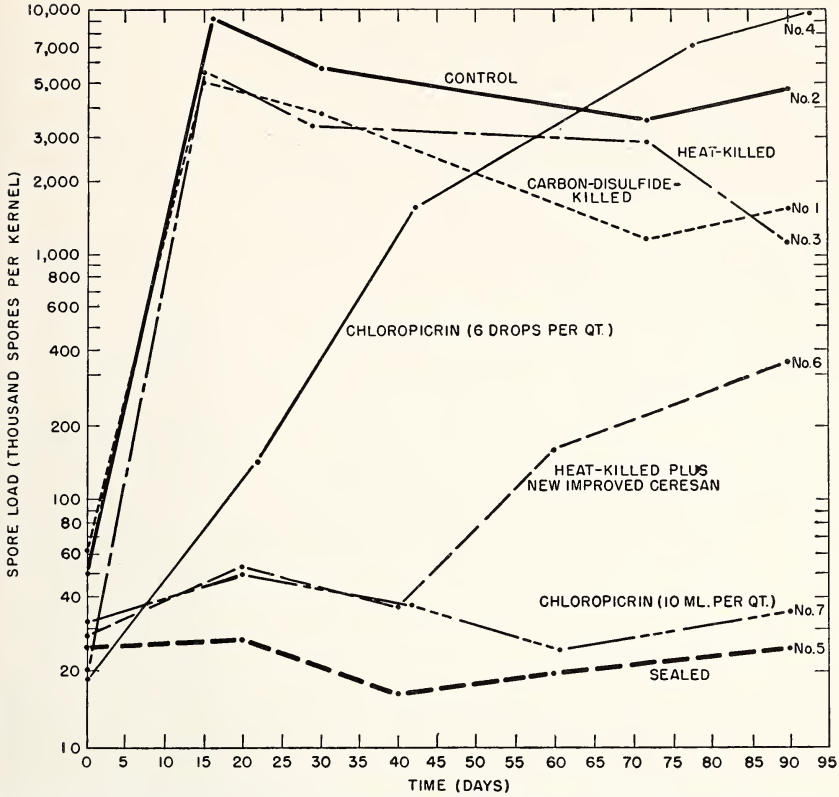


FIGURE 3.—Changes in the spore load of moist wheat in quart glass vacuum flasks after various treatments.

Samples that showed very mild heating in the presence of fungus activity yielded practically pure cultures of *Aspergillus glaucus*. Spore production in these samples rose gradually for about 60 days and in some cases until the termination of the experiment at the end of 90 days.

Application of formaldehyde, toluene, or carbon tetrachloride did not prevent heating (table 2), but the maximum temperatures produced were frequently somewhat lower than those in the untreated (control) flasks of wheat. Wheat treated with toluene quickly lost its ability to germinate. Formaldehyde or carbon tetrachloride also killed the wheat, but not so quickly as did toluene. In addition, wheat was treated with flowers of sulfur, magnetic sulfur, and a 90:10 mixture of sulfur-lime at the rate of 1 gm. per quart of wheat. Because none of these three treatments prevented heating, they are not included in the tables.

Untreated wheat having a moisture content of about 20 percent and a germinating power of 85 percent was compared with samples of the same wheat that had been killed by moist heat or by heavy applications of carbon disulfide. The untreated wheat with a high initial germination heated at approximately the same rate and reached nearly the same maximum temperature as did the samples killed by moist heat or

by excessive carbon disulfide (fig. 2, lines 1 to 3). The spore-load counts indicated that fungus activity was very similar in the 3 types of samples (fig. 3, lines 1 to 3). A maximum of 9,067,750 spores per kernel was observed on the untreated wheat. This figure compares with maxima of 5,470,000 and 5,071,300 spores per kernel for the moist-heat- and carbon-disulfide-killed wheat samples, respectively. The progressive development of fungus growth on wheat pretreated with carbon disulfide is illustrated in figure 4.

TABLE 2.—*Effect of various chemicals on heating and spore loads of viable wheat of various moisture contents stored in cotton-stoppered vacuum flasks*

| Moisture content of grain (percent) | Maximum temperature increment with indicated treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | None (control) | Formaldehyde (1 pound of 1:1 solution per 40 bushels) | Carbon tetrachloride (6.25 ml. per quart) | Toluene (1 ml. per quart) | Chloropicrin per quart | | |
| | | | | | 2 drops | 4 drops | 6 drops |
| | ° *C.* | ° *C.* | ° *C.* | ° *C.* | ° *C.* | ° *C.* | ° *C.* |
| 15.7 | 5. 88 | 5. 08 | | | | | |
| 16.9 | 6. 25 | 6. 38 | 6. 25 | 4. 50 | | | |
| 17.0 | 4. 25 | | 4. 00 | 3. 50 | | | |
| 17.5 | 7. 75 | 8. 25 | | | | | |
| 18.9 | 18. 63 | 17. 12 | | | | | |
| 19.4 | 21. 25 | | | | 8. 50 | 6. 50 | 7. 00 |
| 19.5 | 16. 75 | | 15. 50 | 16. 75 | | | |
| 19.9 | 22. 00 | | 16. 50 | 20. 25 | | | |
| 20.0 | 26. 25 | 19. 75 | | | | | 9. 12 |

| Moisture content of grain (percent) | Maximum spore load with indicated treatment (in thousands) | | | | | | |
|---|---|---|---|---|---|---|---|
| | None (control) | Formaldehyde (1 pound of 1:1 solution per 40 bushels) | Carbon tetrachloride (6.25 ml. per quart) | Toluene (1 ml. per quart) | Chloropicrin per quart | | |
| | | | | | 2 drops | 4 drops | 6 drops |
| | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* |
| 15.7 | 1, 405 | 1, 866 | | | | | |
| 16.9 | 6, 820 | 3, 193 | 1, 118 | 218 | | | |
| 17.0 | 110 | | 36 | 26 | | | |
| 17.5 | 6, 826 | 8, 400 | | | | | |
| 18.9 | 16, 140 | 24, 000 | | | | | |
| 19.4 | 25, 600 | | | | 5, 660 | 15, 000 | 23, 000 |
| 19.5 | 8, 050 | | 15, 980 | 6, 750 | | | |
| 19.9 | 5, 000 | | 6, 350 | 1, 200 | | | |
| 20.0 | 8, 780 | 7, 410 | | | | | 9, 666 |

TABLE 3.—*Effect of various chemicals on heating and spore loads of dead wheat of various moisture contents stored in cotton-stoppered vacuum flasks*

| Moisture content of grain (percent) | Maximum temperature increment with indicated treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Moist heat (120° C.) plus— | | | | Chloropicrin | |
| | None (living control) | No chemical (dead control) | New Improved Ceresan[1] (0.48 gm. per quart) | Carbon tetrachloride (5 ml. per quart) | Carbon disulfide (saturated for 24 hours; killed) | 10 ml. per quart | 10 ml. per quart (exposed for 24 hours; washed in hot water and dried) |
| | ° C. | ° C. | ° C. | ° C. | ° C. | ° C. | ° C. |
| 18.4 | | | | | | 1. 50 | |
| 18.6 | 20. 25 | 20. 50 | 2. 00 | | | | |
| 19.2 | 19. 00 | 20. 00 | | 6. 00 | | | |
| 19.3 | | | | | 24. 25 | | 2. 25 |
| 19.4 | | | | | | 2. 50 | |
| 20.0 | 20. 37 | 20. 00 | | | 23. 25 | 1. 25 | |
| 20.1 | | | | | 24. 00 | | 6. 75 |
| 20.2 | | | | | | 2. 00 | |
| 20.3 | 21. 25 | 23. 50 | | | | | |

| Moisture content of grain (percent) | Maximum spore load with indicated treatment (in thousands) | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Moist heat (120° C.) plus— | | | | Chloropicrin | |
| | None (living control) | No chemical (dead control) | New Improved Ceresan[1] (0.48 gm. per quart) | Carbon tetrachloride (5 ml. per quart) | Carbon disulfide (saturated for 24 hours; killed) | 10 ml. per quart | 10 ml. per quart (exposed for 24 hours; washed in hot water and dried) |
| | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* | *Number* |
| 18.4 | | | | | | 38 | |
| 18.6 | 3, 960 | 11, 160 | 360 | | | | |
| 19.2 | 2, 440 | 6, 900 | | 7, 220 | | | |
| 19.3 | | | | | 12, 220 | | 1, 040 |
| 19.4 | | | | | | 133 | |
| 20.0 | 9, 067 | 5, 470 | | | 5, 071 | 86 | |
| 20.1 | | | | | 6, 660 | | 6, 400 |
| 20.2 | | | | | | 80 | |
| 20.3 | 2, 810 | 7, 933 | | | | | |

[1] 5 percent ethyl mercuric phosphate.

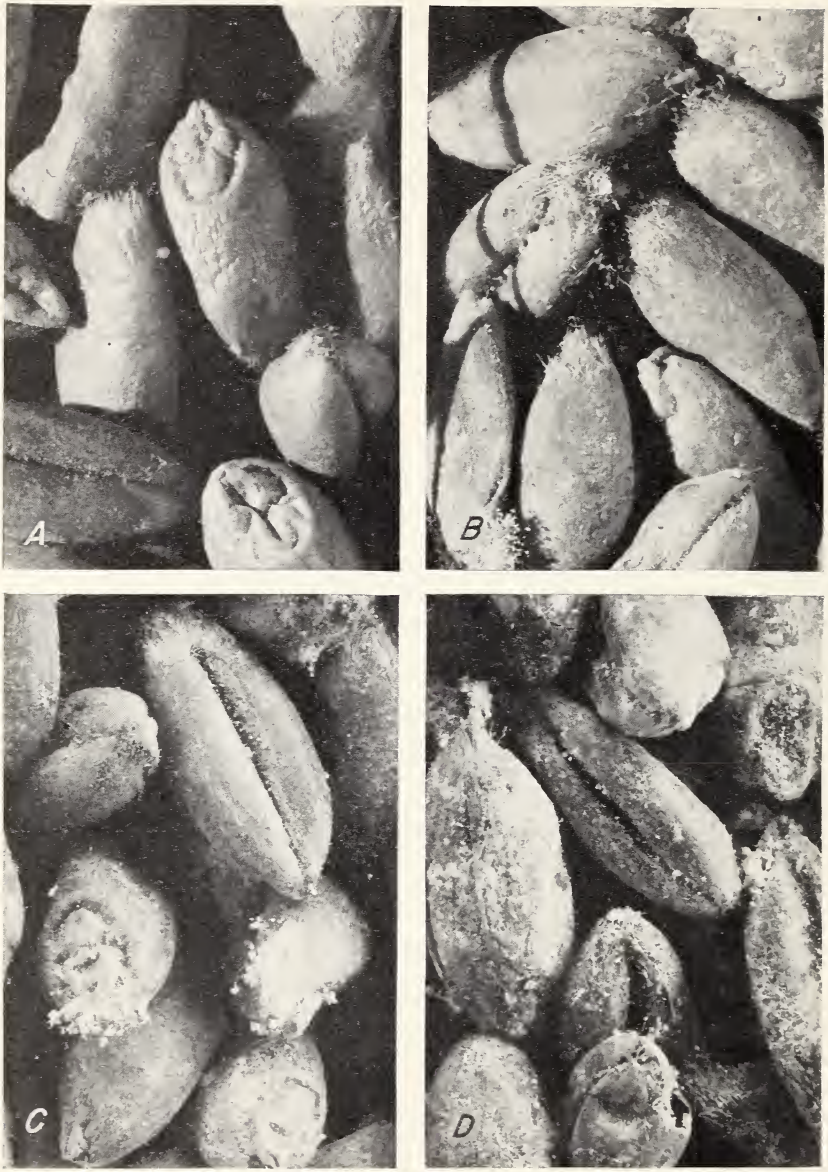


FIGURE 4.—Wheat of 20-percent moisture content pretreated with a heavy application of carbon disulfide to kill the embryos and then stored in cotton-stoppered flasks for various periods: *A*, 9 days; *B*, 30 days; *C*, 72 days; *D*, 90 days. × 6.

The wheat that had been killed by a heavy application of chloropicrin did not heat (fig. 2, line 7, and table 3). Observation showed no increase in spore load and failed to indicate any fungus activity whatsoever. These results may be compared with those obtained when wheat that had been killed by moist heat was mixed with New Improved Ceresan (5 percent ethyl mercuric phosphate) at a rate of 0.48

gm. per quart (½ ounce per bushel). This wheat also failed to heat, as indicated in figure 2, line 6. A slight, but not highly significant, increase in spore load was obtained, a maximum of about 360,000 spores per kernel being reached at the conclusion of the experiment (fig. 3, line 6).

Wheat treated with chloropicrin at a rate of 6 drops per quart heated slightly in vacuum flasks (fig. 2, line 4), but the maximum temperature increment of 9.1° C. was somewhat less than half that produced in the untreated dead and living wheat discussed previously. *Aspergillus glaucus* was the only organism present in this lot. This fungus was apparently able to survive the chloropicrin treatment in its ascosporic stage, whereas conidia of the same fungus succumbed to the treatment. A maximum of 9,666,000 spores per kernel was observed on these samples at the end of 90 days (fig. 3, line 4).

In sealed vacuum flasks, wheat of a 19.5-percent moisture content and an 85-percent germinating capacity did not heat (fig. 2, line 5) and no fungus activity was observed (fig. 3, line 5). Bacteria, however, were plentiful. This wheat quickly became sour and lost its power to germinate.

No mold growth or heat production was observed when wheat of a 14.3-percent moisture content or less was stored in cotton-stoppered vacuum flasks.

When wheat previously killed by moist heat at 120° C. was treated with carbon tetrachloride and placed in vacuum flasks at room temperatures, a marked reduction in heating was noted (table 3) although spore-load studies indicated no reduction in fungus activity. Since this is the only case in which heating was not observed in the presence of fungus activity, it is thought that defective thermocouples or flasks must have been responsible. No significance is attached to this low rate of heating, especially in view of the results noted on viable wheat (table 2).

Treating moist dead wheat with New Improved Ceresan at the rate of 0.48 gm. per quart almost completely inhibited heating and fungus activity. Treated and untreated moist, dead wheat stored in sealed containers failed to heat, but it became sour.

## Relation of Fungus Respiration to Heat Production

The data just presented indicate that heat production in moist stored wheat is almost entirely, if not completely, due to the growth of certain fungi and that heat production is correlated with the spore load on the grain. Total spore load, however, is only a rough measure of fungus activity, because reproduction of the various organisms studied may proceed under different conditions or at different rates. A record of the respiration of fungi on wheat is a much better measure of their activity. In an extreme case, for example, if fungi of the Mycelia-Sterilia group alone were present, the respiration readings would measure their activity whereas spore-load counts would reveal nothing about it.

A series of experiments was designed to determine the relation between the growth of fungi on heat-killed wheat, as evidenced by oxygen respiration and heating, and changes in fat acidity and spore load of similar wheat held in vacuum flasks. The results of the investigation of oxygen respiration and the observed rate of increasing temperature

for the uninoculated control wheat and for each series of inoculations, for two separate experiments, together with the calculated temperature increment derived from the respiration data, are presented in figure 5. Changes in fat acidity and spore load, heating in the vacuum flasks, and the maximum rate of oxygen consumption in the respirometers are recorded in table 4.

In the first experiment the sterile dead control wheat (fig. 5, *A*), which had a moisture content of 20.0 percent, did not heat. No oxygen uptake was observed in these samples. Spore-load and fat-acidity values remained constant within the limits of experimental error for each (table 4). The initial and final spore loads were 1,000 and 970 spores per kernel, respectively.

TABLE 4.—*Changes in fat acidity and spore load and maximum rates of oxygen consumption and heat production caused by fungus growth on autoclaved moist wheat at 28° C.*

| Experiment and inoculum | Fat-acidity value [1] | | Spore load (in thousands) | | | Oxygen consumption maximum in respirometers | Heating | |
|---|---|---|---|---|---|---|---|---|
| | | | | Final | | | Maximum calculated from respirometers | Increment observed in vacuum flasks |
| | Initial | Final | Initial | Respirometers | Vacuum flasks | | | |
| | | | *Number* | *Number* | *Number* | *Mm.³ per gm. dry weight per hour* | ° *C.* | ° *C.* |
| Experiment 1: | | | | | | | | |
| None | 23 | 20 | 1. 0 | 0. 97 | ------ | 5. 5 | 0 | 0. 8 |
| *Aspergillus candidus* | 24 | 240 | 8. 5 | 2, 285 | 3, 250 | 85. 5 | 19. 5 | 15. 6 |
| *A. niger* | 27 | 117 | 7. 3 | 6, 090 | 7, 500 | 57. 0 | 12. 8 | 15. 3 |
| *A. flavus* | 24 | 73 | 3. 6 | 2, 075 | 1, 779 | 103. 0 | 28. 8 | 13. 7 |
| *A. glaucus* [2] | 27 | 119 | 8. 6 | 2, 290 | 2, 210 | 51. 5 | 11. 8 | 11. 9 |
| Experiment 2: | | | | | | | | |
| None | 23 | 20 | . 6 | 2. 65 | 2 | . 9 | 0 | . 9 |
| *Aspergillus candidus* | 25 | 216 | 28. 0 | 2, 290 | 3, 600 | 112. 7 | 24. 1 | 15. 2 |
| *A. niger* | 20 | 99 | 16. 6 | 6, 090 | 6, 833 | 57. 4 | 15. 4 | 8. 0 |
| *A. flavus* | 24 | 76 | 2. 2 | 2, 000 | 2, 500 | 84. 7 | 21. 8 | 4. 7 |
| *A. glaucus* [3] | 27 | 99 | 9. 0 | 2, 390 | 2, 700 | 41. 5 | 10. 9 | 2. 3 |

[1] Milligrams of potassium hydroxide per 100-gm. sample (dry-weight basis).
[2] Reproduction by conidia.
[3] Reproduction by asci.

When a sample of the same lot of wheat was inoculated with a pure culture of *Aspergillus candidus*, rapid heating was observed, reaching a maximum increment of 15.6° C. above room temperature (fig. 5, *B*). This is similar to the heating observed in earlier experiments when this fungus predominated (p. 10). A rapid increase in the rate of oxygen consumption occurred simultaneously with heat production. A peak consumption of 85.5 mm.³ of oxygen per gram of dry weight

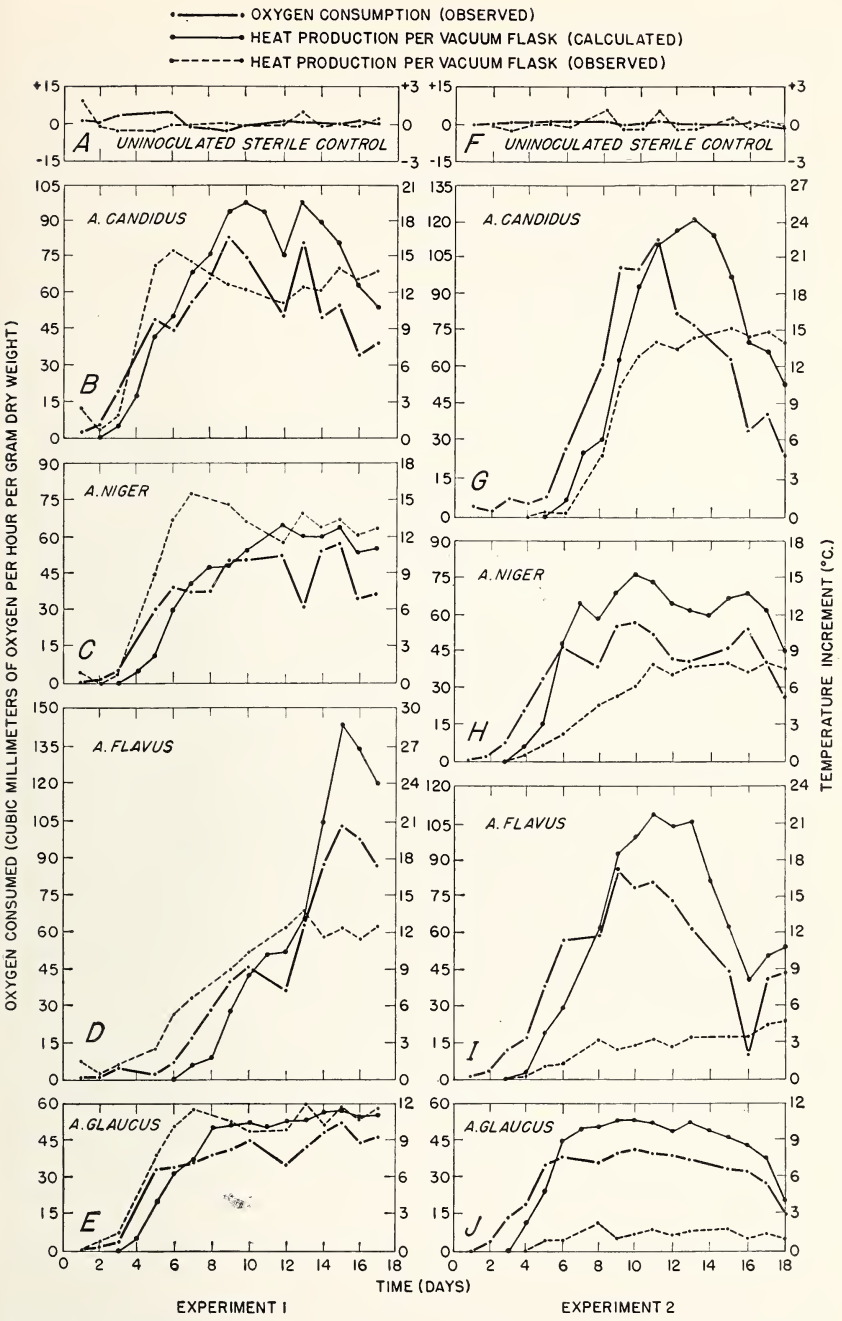


FIGURE 5.—Relation between respiratory activity of certain species of *Aspergillus* growing on sterilized dead wheat and the calculated and observed temperature changes of similar wheat stored in vacuum flasks: *A–E*, Experiment 1, wheat of 20.0-percent moisture content; *F–J*, experiment 2, wheat of 20.3-percent moisture content.

per hour was observed on the ninth day. Spore loads on the samples in the respirometers increased during the 17 days of the experiment from 8,500 to 2,285,000, while the comparable samples in the vacuum flasks increased their spore loads to 3,250,000 spores per kernel. The fat acidity increased from an initial value of 24 to a maximum of 240, indicating a rapid break-down of fats into free fatty acids.

The wheat inoculated with *Aspergillus niger* also showed a temperature increase to an increment maximum of 15.3° C., but the maximum consumption of oxygen was only 57 mm.$^3$ per gram of dry weight per hour (fig. 5, *C*). Spore load in the respirometers increased from 7,300 to 6,090,000 and in the vacuum flasks from 7,300 to 7,500,000 spores per kernel. The fat-acidity value increased from 27 to 117.

*Aspergillus flavus* produced both a high rate of oxygen respiration and a vigorous heating (fig. 5, *D*). A temperature-increment maximum of 13.7° C. was reached on the thirteenth day, and a maximum consumption of 103 mm.$^3$ of oxygen per gram of dry weight per hour was observed on the fifteenth day. The spore load on the wheat in the respirometers increased from 3,600 to 2,075,000, while that of similar wheat in the vacuum flasks reached 1,779,000 spores per kernel and the fat-acidity value increased from 24 to 73.

In the samples inoculated with *Aspergillus glaucus* numerous conidia, but no ascospores, were produced. A temperature-increment maximum of 11.9° C. was noted. The maximum consumption of oxygen was 51.5 mm.$^3$ per gram of dry weight per hour (fig. 5, *E*). The spore load on the wheat in the respirometers increased from 8,600 to 2,290,000 spores per kernel, while that in the vacuum flasks reached 2,210,000 spores per kernel and the fat-acidity value increased from 27 to 119.

In the second experiment the sterile dead control wheat, which had a moisture content of 20.3 percent, again gave no indication of respiratory activity or of heating (fig. 5, *F*). Spore-load and fat-acidity values remained constant during the 18 days of observation.

When the inoculum was *Aspergillus candidus*, respiration was rapid and the wheat heated quickly. The maximum oxygen consumption was 112.7 mm.$^3$ per gram of dry weight per hour on the eleventh day, while the peak temperature increment of 15.2° C. was reached on the fifteenth day in the vacuum flasks (fig. 5, *G*). Rapid increases in both spore load and fat acidity were noted.

The wheat inoculated with *Aspergillus niger* had a maximum respiratory rate of 57.4 mm.$^3$ of oxygen per gram of dry weight per hour on the tenth day. The temperature-increment maximum was 8° C. recorded on the fifteenth day (fig. 5, *H*). The fat-acidity and spore-load values increased greatly during the experimental period. The maximum fat-acidity value observed at the end of 18 days was 99, and the maximum spore load in vacuum flasks was 6,833,000 spores per kernel.

*Aspergillus flavus* caused a maximum oxygen consumption of 84.7 mm.$^3$ per gram of wheat (dry weight) per hour, but comparatively little heating was noted, the maximum temperature increment in the vacuum flasks being only 4.7° C., observed on the eighteenth day (fig. 5, *I*). Fat-acidity values changed from 24 to 76 and the spore load from 2,200 to 2,500,000 in the vacuum flasks.

*Aspergillus glaucus* produced an abundance of ascospores in this experiment; very little heating was noted although a moderate rate of

respiration was maintained (fig. 5, *J*). The temperature-increment maximum was 2.3° C., while the maximum oxygen consumption was 41.5 mm.³. The fat-acidity value increased in the vacuum flasks from 27 to 99, and the spore load in the same flasks increased from 9,000 to 2,700,000 spores per kernel.

The condition of the wheat at the conclusion of the second experiment in Warburg flasks in which respiration was measured is shown in figure 6.

It seems advisable to compare the observed daily temperature increments with the calculated temperature increments expected from the observed respiratory activity. The calculation of the heat liberated in respiration is based empirically on the combustion of sucrose, according to the following formula:

$$C_{12}H_{22}O_{11}+12O_2\rightarrow 12CO_2+11H_2O+1{,}349.6 \text{ kg. cal.}$$

On this basis 5.0208 gm. calories would be liberated for every cubic centimeter of oxygen absorbed.

The calculation of the heat liberated by wheat stored in quart vacuum flasks was based on the rate of respiration as determined in the Warburg respirometers. Earlier tests indicated that a quart-capacity vacuum flask would hold, without packing, about 618 gm. of soft red winter wheat of 20.0-percent moisture content and containing 494.4 gm. of dry matter. The specific heat of wheat of 20.3-percent moisture content was found to be 0.39 calorie per degree centigrade, a value in close agreement with 0.397 calorie per degree centigrade obtained by Winkler and Geddes (*44*). The average thermal capacity of the vacuum flasks was found to be about 1 calorie per degree centigrade.

From these data it was found that for every cubic millimeter of oxygen absorbed per gram of material (dry weight) in the Warburg respirometers per hour, a total of 59.46 gm. calories was produced in each vacuum flask per day; however, 193.5 gm. calories were necessary to raise both flask and contents 1° C. Therefore, an observed respiratory rate of 1 mm.³ of oxygen per gram of wheat (dry weight) per hour was equivalent to the production of 0.307° per vacuum flask of wheat per day. With this number as a basis for calculating heat production and with the calibration curves presented in figure 1 as a basis for calculating heat lost by radiation, curves were constructed for the theoretical temperature output of the fungi growing on sterile dead wheat in the vacuum flasks. These values are presented as the calculated daily temperature increments.

It should be borne in mind that in calculating heat production there are several possible sources of error, the most important of which are the assumptions (1) that all the vacuum flasks contained 618 gm. of wheat at 20-percent moisture and (2) that the thermal capacity of all the vacuum flasks was 1 calorie per degree, whereas, in fact, individual flasks were found to range from 0.758 to 1.250 gm. calories per degree. Furthermore, in experiment 1 (fig. 5, *A* to *E*) no respiration readings were obtained on the eleventh day. The rates for this day were calculated by averaging the readings on the tenth and twelfth days. The sudden break in the curves for respiratory rate at this point is due undoubtedly to the limitation of oxygen because of failure to aerate the respirometers on the eleventh day. After aeration on the twelfth day, recovery of respiratory activity was slow. In experi-

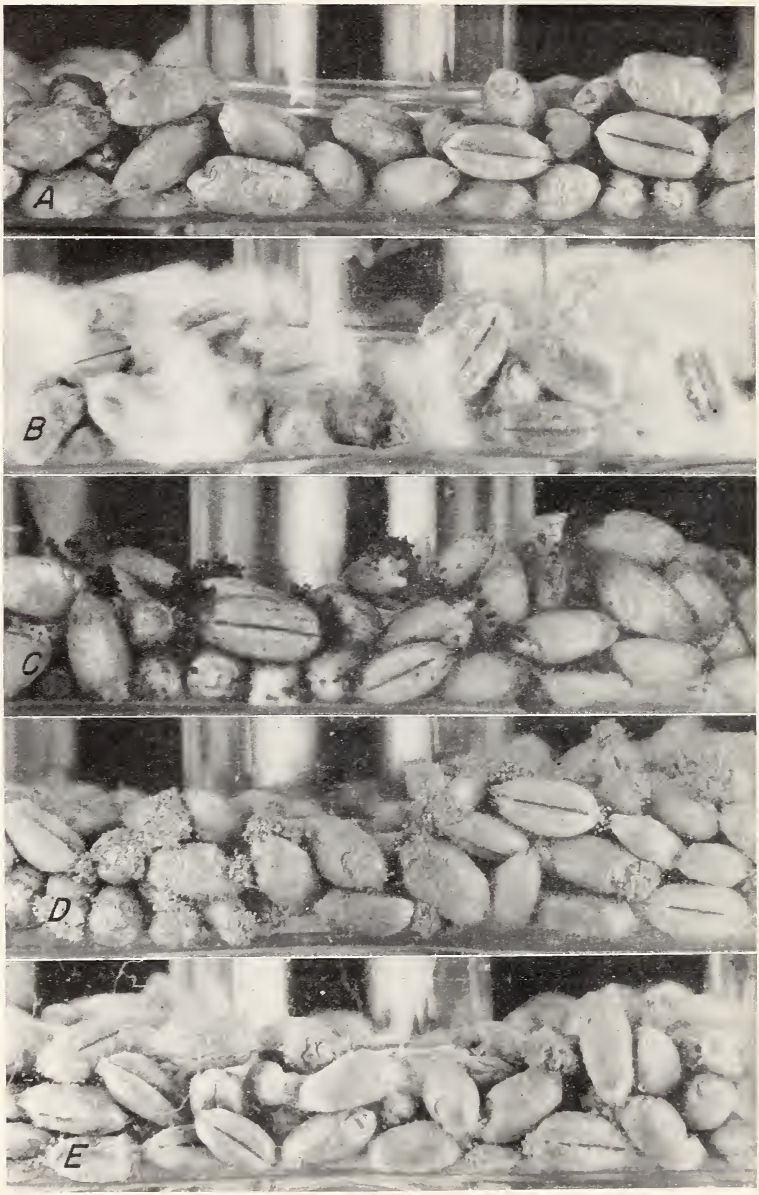


FIGURE 6.—Wheat in Warburg respirometers at conclusion of respiration experiment 2: *A*, Uninoculated, sterile dead wheat (control); *B*, inoculated with *Aspergillus candidus*; *C*, inoculated with *A. niger*; *D*, inoculated with *A. flavus*; *E*, inoculated with *A. glaucus*. × 2.2.

ment 2 (fig. 5, *F* to *J*), the same applies, because of lack of readings on the seventh and fourteenth days. It is quite probable that, if the respirometers had been aerated every day, there would have been no such breaks in respiratory rate and the calculated heat production would be somewhat greater.

Close agreement between the calculated and the observed temperature increments should not be expected. The calculated values are based on readings of oxygen consumption in respirometers, whereas the observed data are taken from similar material in vacuum flasks. The calculated values are presented to show only that all the heating actually observed can be accounted for on the basis of energy released by the respiration of fungi on dead wheat. Temperature and oxygen tension were held at nearly constant levels, with the exceptions noted, in the respirometers, but they were allowed to vary spontaneously in the vacuum flasks.

The following estimate of the respiratory rate of soft red winter wheat of various moisture contents at 28° C. is available from observations of Shirk and Appleman (*37*) and from unpublished work of the writers:

| Total moisture content: | *Rate of respiration (mm.³ of $O_2$ consumed per gram of dry weight per hour)* |
|---|---|
| 17.6 percent | 1.50 |
| 18.1 percent | 2.00 |
| 19.0 percent | 6.05 |
| 21.3 percent | 8.50 |
| 23.0 percent | [1] 13.00 |
| 27.7 percent | [1] 37.70 |
| 29.4 percent | [1] 49.50 |

[1] Calculated from data of Shirk and Appleman (*37*) on rate per gram of fresh weight.

These observations on respiratory rate were made at an interval between 25 and 36 hours after moisture adjustment. Numerous tests have indicated that, in general, wheat will reach an even distribution of moisture in 25 hours after the addition of water, provided it is agitated thoroughly a few times during this period.

The activity of fungi present on wheat is still at a minimum 36 hours after moisture adjustment, but it begins to increase shortly thereafter.

By calculating, from the data just presented, the energy released in the respiration of a quart of wheat in a vacuum flask, it was found that a moisture content of about 18.5 percent would be required before the rate of energy release would be sufficient to cause any increase in temperature.

## DISCUSSION

The variety of fungi found on wheat is not surprising when it is realized that the glumes of the wheat plant give little protection to the developing caryopses while the plants are exposed to air-borne and soil-inhabiting fungi in the field. Furthermore, glumes and awns serve as effective traps for air-borne and rain-spattered spores, which may be easily transferred to the grain during threshing.

Although dry No. 1 wheat may carry several thousand spores per kernel (table 1), it is probable that only a small proportion of these are capable of germination and growth. The relatively low spore load on heating wheat from the experimental bins may have been caused by the jarring of the spores from the kernels while the samples of wheat were being shipped and handled. This suggests that all spore-load determinations were conservative, because many spores were lost in the sampling and subsequent handling of the wheat before the spores were counted.

Heating curves obtained in these experiments do not support the two-phase hypothesis of Cohn (*12*), but strongly suggest the conclusion

that the growth of micro-organisms is the main, if not the sole, cause of heat production in stored moist wheat. This is emphasized by the fact that wheat previously killed by moist heat or other means that permitted the later growth of fungi heated at about the same rate and reached about the same maximum temperature as did wheat not so treated. Furthermore, the fungi usually showed considerable growth activity by the third day in moist storage and this was reflected in rising temperatures.

The data obtained on heat production in dead wheat treated with 0.48 gm. of New Improved Ceresan per quart of grain indicate that heating of moist wheat may be partly prevented or controlled by the admixture of fungicides. This compound or other chemicals containing poisonous substances, however, could not be used on wheat that is intended for human or animal consumption. Chemical agents used for controlling or preventing heating in wheat should be nonpoisonous and practically odorless, have a low rate of volatilization, and should not injure the germination or milling and baking qualities of the wheat.

The results of storing moist wheat in airtight vacuum flasks agree with those presented by Dendy and Elkington (*14*).

The experimental data presented in this circular on respiration of viable wheat obtained before contaminating micro-organisms became active and on the respiration of certain fungi growing on dead wheat strongly support the findings of Gilman and Barron (*16*), Leach (*24*), Ramstad and Geddes (*34*), and others.

The activity of fungi present on wheat is stimulated by moisture levels slightly above 14 percent, with various species commencing activity at different moisture levels, as shown by Koehler (*22*). The investigations reported by Kelly and associates (*21*) indicated that heating in large bulks of grain can occur at moisture levels somewhat lower than those found necessary to obtain maximum activity in the quart vacuum flasks used in the experiments herein.

The curves showing the correlation of oxygen consumption with heat production in wheat samples are very similar to those obtained by Carlyle and Norman (*7*), in a comparison of carbon dioxide evolution and heat production in moist straw inoculated with certain thermophilic bacteria.

The observed and calculated temperature-increment curves presented in figure 5 show that, with a possible exception in *C*, all of the heat production observed could have been produced by the respiration of the fungi growing on a substrate of dead soft red winter wheat.

The maximum temperature increments observed for the fungi used in these experiments vary, because in vacuum flasks the optimum temperatures for the growth of any particular organism are soon exceeded. *Aspergillus glaucus*, according to Thom and Church (*40*) and Thom and Raper (*42*), grows best at, or slightly below, blood temperature, while *A. niger* and *A. flavus* also grow well at 37° C. *A. candidus* has frequently been isolated from heating wheat and is tolerant of temperatures of 50° to 55°.

Differences in heating caused by the same organism in the two experiments presented may be partly accounted for on the basis of observations reported by Hurd (*18*) in 1921 and by Mead, Russell, and Ledingham (*26*) in 1942 and indicating that the percentage of embryo exposure (broken seed coats) is a factor in the quantity of mold growth and

heat production that may be observed. A third factor limiting heat production in vacuum flasks is oxygen tension. It was found that a flask containing 618 grams of wheat at 20.0-percent moisture would have about 353 cc. of air space, or roughly 74.0 cc. of available oxygen. This supply would quickly become exhausted and gaseous exchange through a loose-cotton stopper would be insufficient to supply all the oxygen needed to maintain the full possible respiratory rate. The results of several investigations (*19*, *20*, *43*, *44*) on the influence of aeration on heat production in stored moist organic materials indicate that somewhat higher temperature increments would have been obtained in the studies herein reported if the flasks had been aerated.

In relation to the heating of moist organic materials, Norman, Richards, and Carlyle (*32*) pointed out that certain constituents of the substrate decompose with the evolution of more heat than is evolved in the decomposition of other constituents. The rate of heat evolution, therefore, would depend partly upon the substrate constituents oxidized by the various fungi and the relative rates at which those constituents were decomposed.

## SUMMARY AND CONCLUSIONS

Many species of fungi were isolated from stored soft red winter, hard red winter, and hard red spring wheats. Species of *Aspergillus* and *Penicillium* usually predominated in isolations from the surface of wheat kernels, while species of *Alternaria* and *Aspergillus* were commonly found in the interior.

Studies on spore load indicated that No. 1 grade soft red winter wheat carried 3,000 to 57,000 spores per kernel. Wheat that had heated frequently carried several million spores per kernel.

Investigations on the effect of fungi on the heating of wheat in storage showed that—

*Aspergillus candidus* usually was involved in actively heating wheat. Temperature increments of 20° to 26.25° C. were attained in wheat infested with this fungus.

*Aspergillus glaucus* that was reproducing by asci was found to predominate in wheat samples that heated only moderately.

Moist wheat, treated to prevent the action of respiratory enzymes and allowed to become reinfested with spores in the atmosphere, was found to heat at the same rate and to reach the same temperature maximum as untreated moist wheat.

Moist wheat of high germinating capacity treated with New Improved Ceresan did not heat, and only slight fungus activity was evident. This further indicated that respiration in the wheat kernel is unimportant in producing the heat that developed in improperly stored wheat.

Moist wheat killed by a heavy application of chloropicrin failed to heat, and no fungus activity was observed.

High-moisture wheat stored in sealed vacuum containers did not heat. Bacteria, but no fungi, were isolated from these samples.

The Warburg manometer was used to measure the oxygen absorbed in the respiration of three types of wheat: (1) Living wheat on which the contaminating fungi were inactive; (2) sterile dead wheat; and (3) previously sterilized dead wheat that had been inoculated with pure cultures of certain species of *Aspergillus*. The respiration of the

fungi was compared with their ability to produce heat on aliquot samples of wheat stored in quart vacuum flasks. Steam-sterilized moist wheat did not respire, and heating was not observed. When moist sterilized wheat was inoculated with *Aspergillus candidus*, *A. flavus*, or *A. niger*, high rates of respiration and heat production were noted. *A. glaucus* sometimes produced different rates of respiration and heating on this moist dead wheat. These differences were associated with the type of spores produced. Production of ascospores was associated with moderate oxygen uptake and very little heat production. The appearance of conidia, on the other hand, coincided with higher respiratory measurements and more heating.

The data indicate that the heating of moist wheat in storage can be entirely accounted for by the energy released in the respiration of the fungi present on and in the wheat kernels.

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